Scientific Journal for Damietta Faculty of Science 6 (2) 2016, 132-143 ISSN Print 2314-8594
ISSN Online 2314-8616

Clinical Significance of CD200 and CD56 in Patients with Acute Lymphoblastic Leukemia

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Received: 3 Sept. 2016 /Accepted: 17 November 2016
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Abstract

To analyze CD200 and CD56 expression by flow cytometry in acute lymphoblastic leukemia patients and whether their overexpression had prognostic impact individually and in combination with each other. Seventy newly diagnosed patients were assessed, of whom 27 were adult ALL and 43 pediatric ALL. Forty seven of 70 cases (67%) showed CD200 expression, and 7 cases (10%) showed CD56 expression but only 3 cases (4.3%) had expression for CD200+ and CD56+. Significance differences were found between CD200& CD56 expression in whole ALL patients group compared to control group (P<0.0001and P<0.001respectively). Splenomegaly and lower hemoglobin and platelet were more frequently observed in CD200+ patients whose also associated with significant increase of myeloid marker CD34 frequency. There were significant differences in overall survival (P= 0.042, P= 0.006) and disease-free survival (P= 0.005, P= 0.002) between the CD200+ and CD200- patients in total ALL patients and adult ALL . Whereas, in pediatric ALL OS of CD200+ patients 35.7% compared to 86.2% in CD200-, P= 0.032 but DFS showed non-significant difference (P=0.099). On the other hand, CD56+ patients had lower complete remission rate (14.3% vs. 63.5%, P= 0.018). CD56+ had significant influence on overall than those of CD56- (28.6% with mean 4.7 months vs. 41.1% with mean=11.8 months, P= 0.003) and disease free survival (40% with mean=6.26months vs. 54.9% with mean=14.16, P = 0.006). In respect to combination of two CDs positive had very inferior OS and DFS (mean =0.533 month and 0.150 month). In addition to, increased frequency of CD34 was associated with CD200+, CD56+ patients.

Keywords: *Lymphoblastic Leukemia, Gene Expression, Immuno-phenotyping*

Introduction

Acute lymphoblastic leukemia is a serious type of malignancies that originates from the development and differentiation of the early version of lymphocytes in the bone marrow and has an Acute lymphoblastic leukemia is a serious type of malignancies that originates from the development and differentiation of the early version of lymphocytes in the bone marrow and has an

annual increase of 1.4%, in the incidence worldwide. The highest incidence was observed in the ages between 1-4 years with a marginal predominance of males over female **(Timothy *et al.*, 2016)**.

CD200 is a trans-membrane cell surface glycoprotein encoded by a gene residing at chromosome 3q12.(McCaughan *et al.*, 1987). It is belonging to the type-I immunoglobulin superfamily and also known as MOX-2" (Wright *et al.*, 2001). It is related to the B7 family of co-stimulatory receptors, with two extracellular domains, a single transmembrane region, and cytoplasmic tail without signal motif (Barclay *et al.*, 2002).

CD200-CD200R interactions have a role in modulated cell mediated immunity with significant lymphocytic involvement (Walker *et al.*, 2013), regulation of myeloid differentiation and or activation , fetal loss, transplant rejection (Gorczynski, 2001) and autoimmunity (Gorczynski *et al.*, 2002) . However , CD200 has a central role in immune tolerance that protect stem cells and other critical tissues from immune damage (Gorczynski *et al.*, 2001).CD200 can induce neuritogenesis and promote neuronal survival in primary neurons through activation of the fibroblast growth factor receptor (FGFR) (Pankratova *et al.*, 2016).

CD200 with its receptor CD200R delivers an inhibitory signal to the target cell in most circumstances (Jenmalm *et al.*, 2006) and imparts an immunosuppressive signal leading to inhibition of macrophage function (Hoek *et al.*, 2000), induction of regulatory T cells (Gorczynski *et al.*, 2005), switching of cytokine profiles from Th1 to Th2 and inhibition of tumor-specific T-cell immunity(Coles *et al.*, 2011) . In leukemia and other malignancies CD200(OX2) act as an immunosuppressive surface protein its expression associated with inhibition of antitumor T cell responses and decreased overall survival of patients (Shannon K Oda1 2016). CD200 suppresses the immune system's response to vaccines, and that blocking CD200 could improve the efficacy of cancer immunotherapy (Xiong *et al.*, 2016).

CD200 expression on human malignancies is associated with tumor progression, and predict worse overall survival for various forms of leukemia patients **(Rygiel *et al.*, 2012)**. AML patients with CD200 high were shown to have inferior survival compared with those CD200low and increased risk of relapse **(Atfy M, 2015)**.

CD56 is a neural- cell adhesion molecule (N-CAM) that known as a marker of natural killer cells (NK) (Farag et al., 2003). It expressed on a subset of normal T cells and occasionally on blasts in T-cell acute lymphoblastic leukemia especially a subgroup of T-ALL patients who did not respond to therapy (Dalmazzo et al., 2009). NK- cells are phenotypically and functionally very similar to T- cells (cytotoxic T-cells) as it arise from T/NK bi-potential common progenitor **(Shibuya *et al.*, 1995).**

High incidence of CNS disease was found in adult ALL patients who expressed CD56 **(Ravandi *et al.*, 2002a) .** The expression of CD56 has been shown to be of prognostic relevance in certain hematologic malignancies as well as it was found an inferior survival in small series of $CD56^+$ T-ALL **(Montero *et al.*, 2003)**. CD56 expression should be regarded as an independent risk factor for ALL with CNS involvement in adults **(Hu *et al.*, 2016)**. Also aberrant co-expression of the NK cell marker CD56, CD117 and CD33 and the stem cell marker CD34 in a patient with T-cell ALL appears to be associated with an unfavorable outcome **(Eren *et al.*, 2016).**

The aim of the present study is the estimation of CD200 and/or CD56 expression in acute lymphoblastic leukemia patients and their prognostic impact.

Materials and Methods

Patients:

Pretreatment bone marrow and peripheral blood specimens were obtained from 70 patients (2-52 years of age) at Mansoura Oncology Center, Mansoura University, Egypt. Lymphoblastic leukemia cases were diagnosed according to the French-American- British criteria and immunophenotypic analysis. Patients

with newly diagnosed acute lymphoblastic leukemia were included in this study subjected to these criteria; laboratory investigations, immune-phenotypic and clinical examination of patients. Complete remission (CR) was considered when bone marrow blast cell counts were <5%.

Immuno-phenotyping

Immuno-phenotypic analyses for ALL patients were performed according to standard techniques on a FACS can flow cytometer. In order to perform IPT of the blasts a broad panel of fluorochrome conjugated monoclonal antibodies (mAbs) were used that included : anti- CD3(PE-HIT3a), CD4(FITC-RPA-T4), CD5(FITC-UCHT2), CD7(FITC-8H8.1), CD8(APC–RPA-T8),CD10(PE–HI10a),CD13(PECY5-Immu103-44), CD14(PE-M5E2),CD19(PE-HIB19), CD20 (PE-2H7), CD22 (PE-HIB22), CD33(FITC-HiM3-4), CD34(PE-581), HLADR(PE-Immu-357) and TDT(FITC-E17-1519). In addition, CD200 (PE- MRC OX-104) and CD56 (FITC- NCAM16.2) mAbs were also used. Samples were stained with monoclonal antibodies against cell surface markers as described before using stain – lyse - wash then direct immunofluorescence technique was done **(Porwit *et al.*, 2015)**. Briefly, 100µl of BM or PB sample were added to 20µl of mAb of each CD, then incubated for 15 min at room temperature. Cell suspensions were washed with phosphate buffer saline (PBS) and 2 ml of 1x BD FACS Lysing solution were added. Vortex and incubate 10 min. at room temperature and then centrifuged for 5 min at 350 rpm. Cells were washed twice with PBS, the cell pellet was re-suspended in 500µl PBS. Then, the sample became ready for acquisition and analysis by flow cytometry. When expression of a marker on the lymphatic blasts ≥ 20 defined as positive.

BCR- ABL detection

Samples were tested for t(9;22) by using real-time quantitative PCR. The assay involves a duplicate real-time amplification reaction for the target and a duplicate real-time amplification reaction for the control. There are two main parts in the test, the amplification reaction is carried out specific for a region of the mRNA of P210, while in the second step, an amplification reaction is carried out specific for a region of the mRNA of ABL using the cDNA produced by the reverse transcription reaction of the RNA extracted from the test samples. System standardization was carried out on Applied Biosystems ABI PRISMTM 7000 series instruments.

Statistical analysis

All statistical analyses were performed using the SPSS software package and Graph Pad Prism (Graph Pad Software, Inc; San Diego, CA) to analyze P values. Data were statistically described in terms of mean ± SE, mean ± SD and mean with range. Overall survival was calculated from the date of first diagnosis to death from any cause. Whereas remission duration was calculated from the time of achievement of CR to time of relapse or death in CR. A probability value (*p* value) less than 0.05 was considered statistically significant.

RESULTS

Patients characteristics

The patients had a wide age range (2-56 years; mean 14.3). The majority 61.4%, were children younger than 18 years with 7.8 mean age and about 38.6%, were adult with mean age 32.7 year (table,1).

Analysis of CD200 expression

ALL Patients with $CD200^-$ expression were significantly associated with male gender. $CD200^+$ Patients were significantly associated with Splenomegaly. Otherwise, no significant associations were found between demographic or clinical data according to CD200 positivity when total ALL patients reclassified into pediatric and adult all patients (table 1).

$CD200^+$ all patients presented with lower hemoglobin and platelet count compared to those with $CD200^-$. Whereas, CD200 positivity in adult ALL patients had not influence in laboratory data (table 2).

Table (1). Pre-treatment characteristics according to CD200 positivity in total ALL patients.

	Total ALL(n=70)				*P*
	$CD200^-$ (N=23)		$CD200^+$(N=47)		
	N	%	N	%	
Age (years)	15.3	2-56	13.5	2-52	0.862
Males	18	75.0	22	47.8	0.029**
Females	6	25.0	24	52.2	
Pallor	12	50.0	24	52.2	0.863
Fever	16	66.7	35	76.1	0.400
Weight loss	9	37.5	13	28.3	0.429
Pain	12	50.0	21	45.7	0.729
Bleeding tendency	4	16.7	12	26.1	0.373
Bone ache	5	20.8	6	13.0	0.493
Splenomegaly	15	62.5	40	86.9	0.031*
Hepatomegaly	21	87.5	36	78.3	0.520
Lymphadenopathy	19	79.2	33	71.7	0.500

N&%, number of pre-treatment ALL patients with each clinical data and their ratio.
*, indicating significant difference.
**, highly significant.

Table (2). laboratory data according to CD200 positivity in total ALL patients.

	Total ALL(n=70)		***P***
	$CD200^-$ (n=23) Mean	**$CD200^+$ (n=47) Mean**	
Total leucocytic count ($X10^9$/L)	23.3 (1.5-520)	26.6 (1.6-374.8)	0.839
Hemoglobin concentration (g/dL)	8.1 (3.8-12.2)	6.9 (3-13.8)	0.046*
Platelet count ($X10^9$/L)	92.3 (7-582)	49.9 (5-186)	0.027**
Peripheral blasts (%)	45 (0-88)	85 (70-95)	0.053
Marrow blasts (%)	88.5 (62-96)	90 (75-98)	0.837
Albumin (g/dL)	4.095 (3.1-5.0)	4.1 (1.8-5.5)	0.931
Bilirubin (mg/dL)	0.8 (0.4-2.0)	0.7 (0.2-4.0)	0.804
ALT (U/L)	18.35 (7-130)	23.85 (8-168)	0.552
AST (U/L)	38.5 (19-211)	34 (14-124)	0.237
Creatinine (mg/dL)	0.97 (0.5-3.9)	0.8 (0.3-8.2)	0.240
Uric acid (mg/dL)	6.37 (1.8-33.2)	4.925 (1.3-14.6)	0.066
LDH (U/L)	1105.85 (176-9373)	616.5 (131-3997)	0.080
Calcium (mg/dL)	8.75 (5.9-12.1)	8.4 (6.1-11.5)	0.216

Cases were assigned to the $CD200^-$ (<20) and CD200+ (>20) subgroups based on independent assessment of CD200 expression by flow cytometry. Data between arches represent range.

Analysis of CD56 expression

There were no significant differences in both the $CD56^+$ and $D56^–$ subgroups concerning sex and clinical presentation. There were high proportion of patients in both groups presented with splenomegaly, lymphadenopathy and hepatomegaly. However $CD56^+$ ALL presented with higher platelet counts compared to the $CD56^–$ patients . This difference was statistically significant in both total ALL patients and pediatric ALL patients (P = 0.024, P = 0.019).

Table (3). Laboratory data according to CD56 positivity in total ALL group.

	$CD56^{-ve}$ (n=63) Mean	**$CD56^{+ve}$ (n=7) Mean**	***P***
Total leucocytic count ($X10^9$/L)	26.6 (1.5 – 520)	18.3 (1.7 – 39.1)	0.236
Hemoglobin concentration (g/dL)	7.17 (3.0 – 13.8)	7.7 6.8- 10.6)(	0.129
Platelet count ($X10^9$/L)	51.5 5 – 582)(	140 26 – 397)(	0.024*
Peripheral blasts (%)	90 62 – 98)(	90 80 – 90) (	0.214
Marrow blasts (%)	70 0 – 98)(	80 55 – 93)(	0.201
Albumin (g/dL)	4.1 2.8 – 5.5)(	3.9 (1.8 - 4.1)	0.076
Bilirubin (mg/dL)	0.8 0.2 – 4.0)(	0.7 0.4 - 1.7)(	0.784
ALT (U/L)	20 7 – 168)(	19 11 – 99)(	0.487
AST (U/L)	37 14 – 211)(	34 (15 – 126)	0.914
Creatinine (mg/dL)	0.85 0.3- 8.2)(	0.6 0.5 – 1.0)(	0.098
Uric acid (mg/dL)	5.4 1.3 – 33.2)(	3.47 (1.8 – 5.9)	0.094
LDH (U/L)	714.9 (190 – 9373)	461 (131 – 7414)	0.564
Calcium (mg/dL)	8.55 5.9 – 11.5)(	8.7 (7.9 – 12.1)	0.473

Expression of CD200 and CD56:

Total ALL patients according to combination of CD200 and CD56 positivity were reclassified into four groups: ALL Patients with $CD200^+$ $CD56^+$, $CD200^+$ $CD56^-$, $CD200^-$ $CD56^+$ and $CD200^-$ $CD56^-$. As in table(4), although, statically no significant associations were found between demographic or clinical data according to CD200, CD56 positivity in total ALL patients , $CD200^+$ $CD56^+$ Patients were associated with more pain and increased frequency of Splenomegaly. Whereas, $CD200^-$ $CD56^+$ patients had male gender prevalence with hepatomegaly.

$CD200^+$ $CD56^+$ patients had the lowest platelet count (53; range 26 – 140) and significant lower LDH activity. Whereas patients with $CD200^-$ $CD56^+$ were characterized by increased platelet count at diagnosis and higher LDH activity compared to other studies groups.

Table (4). Demographic and clinical data according to CD200, CD56 positivity in total ALL patients.

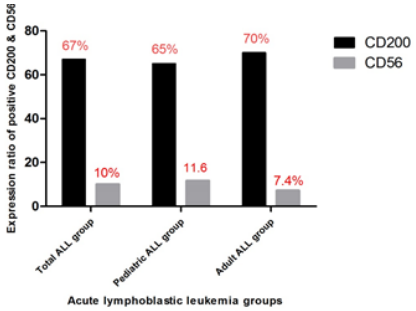
	ALL(n=70)								
	$CD200^-$ $CD56^-$ N=20		**$CD200^+CD56^-$ N=43**		**$CD200^-$ $CD56^+$ N=4**		**$CD200^+CD56^+$ N=3**		***P**
Age(years); mean, range	15.3	2-56	13	2-52	12.9	7-50	14.6	8-35	0.918
N., % of patients	N	%	N	%	N	%	N	%	
Males	15	75	21	48.8	3	75	1	33.3	0.155
Females	5	25	22	51.2	1	25	2	66.7	
Pallor	11	55	22	51.2	1	25	2	66.7	0.725
Fever	13	65	33	76.7	3	75	2	66.7	0.811
Weight loss	8	40	12	27.9	1	25	1	33.3	0.802
Pain	9	45	19	44.2	3	75	2	66.7	0.655
Bleeding tendency	3	15	11	25.6	1	25	1	33.3	0.677
Bone ache	4	20	5	11.6	1	25	1	33.3	0.354
Splenomegaly	14	70	35	81.4	1	25	3	100.0	0.065
Hepatomegaly	17	85	33	76.7	4	100	3	100.0	0.784
Lymphadenopathy	17	85	32	74.4	2	50	1	33.3	0.134

* P value was determined using one way ANOVA test.

Immuno-phenotype

Expression of CD200 was evaluated in 70 pre-treatment acute lymphoblastic leukemia patients. A total of 67 % of all patients were positive for CD200 but only 10% positive for CD56 as well as in relation to age 65% of pediatric ALL patients were positive for CD200 but about 11.6% positive for CD56 . However in adult ALL patients about 70% were positive for CD200 and only 7.4% positive for CD56 (figure,1). Only 3 out of 70 ALL patients in our study have been found positive for both CDs. CD200 positively was observed in 72.5% of B – ALL patients that also 7.8 % positive for CD56 whereas, 60% and 13.3 % of T-ALL patients were positive for CD200 and CD56 respectively figure(2,3). These observations led us to assess the expression of CD200 in relation to FAB classification that summarized in table (5) CD200 more frequency positive in patients with L2 88.1% but only 2.4% CD56+ , as well as 47.6% of L1 positive for CD200 and 19% for CD56 . Although none of L3 express CD200 about 28.6 % positive for CD56.

The immune-phenotypic features were evaluated in all the ALL Patients. Both groups show similar finding, except for CD34+ that had significantly high frequency of positive expression in $CD200^+$.



Fig(1). Expression ratio of CD200 & CD56 positive in acute lymphoblastic leukemia groups.

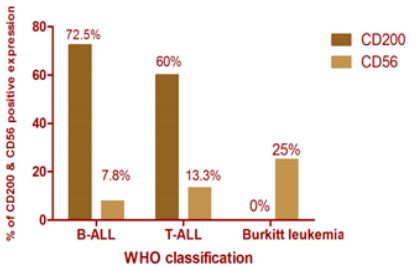


Fig (2). Ratio of expression of CD200 & CD56 positive regarding WHO classification of ALL patients.

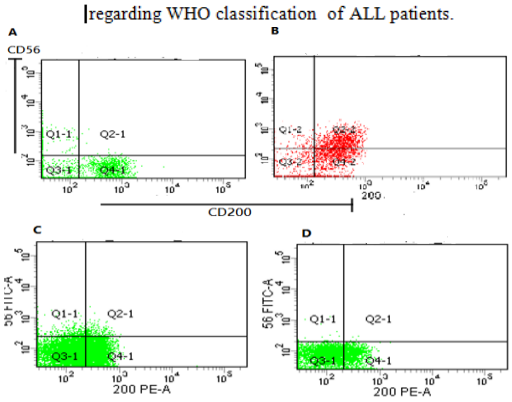


Fig. 3 . Expression of CD200and CD56 in B-ALL. (A)B-ALL patient with CD200 positive and CD56 negative . Whereas,(B)represent B-ALL patients with two positive CDs ($CD200^+$, $CD56^+$). Expression of CD200and CD56 in T-ALL. (C) represent T-ALL patients with high positive CD200 and low positive CD56. Whereas, (D) T-ALL patient with CD200 positive and CD56 negative.

Table (5). Expression of CD200 in FAB classification of total Acute lymphoblastic leukemia patients group.

FAB	No. of cases	No. , % with CD200 expression	No. , % with CD56
L1	21	10 (47.6%)	4 (19%)
L2	42	37 (88.1%)	1 (2.4%)
L3	7	0 (0%)	2 (28.6%)

Expression of CD200 and CD56 in relation to PCR – APL gene translocation

There are six patients out of seventy have Philadelphia t(9;22) positive and 83% of them are CD200 positive but none of them express CD56.In our study only one pediatric case is Philadelphia t(9;22) positive which also CD200 positive but the other four positive CD200 cases from adult ALL group, **fig(5).**

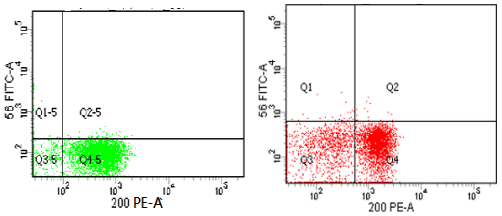


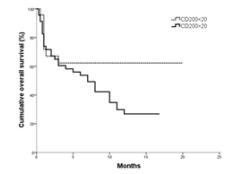
Fig 5. Expression of CD200and CD56 in Philadelphia t(9;22)positive ALL. The patients positive for CD200 but negative for CD56.

Treatment results

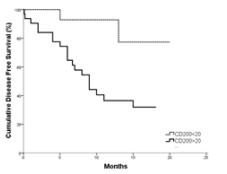
Expression of CD200 had a significant adverse effect in clinical outcome and survival in ALL patients (figure,6) . CR rates did not differ in $CD200^+$ ALL patients compared with $CD200^-$ ALL (54.2 v 60.9 ; 53.3 v 60.7 and 55.6 v 61.1) in total ALL patients , pediatric patients and adult patients respectively.

After a median follow- up of 25 months we find a significant influence of CD200 expression on overall survival (OAS). The cumulative OS % for $CD200^-$ groups compared to $CD200^+$ groups were (62.3 vs 28.7 ; 86.2 vs 35.7 and77.8 vs 35.7) in total ALL patients , pediatric patients and adult patients , as well as the cumulative DFS % were (77.4 vs 34.7 and 75 vs 10.9) in all patients and adult patients but in pediatric ALL patients CD200 did not effect in cumulative DFS % (87.5 vs 49.5, *P =0.099).*

On the other hand, significantly less patients with $CD56^+$expression achieved complete remission compared to $CD56^-$ in total patients .However in pediatric CR rates did not differ regarding CD56. $CD56^+$ patients had an inferior survival compared to those with $CD56^-$ (41.1% vs 28.6 and 50.1 vs 20) and shorter DFS (54.9 vs 39 % and 65.7 vs 25 %) .



OS according to CD200 expression in total ALL patients(P = 0.042).



DFS according to CD200 expression in total ALL patients (P =0.006)

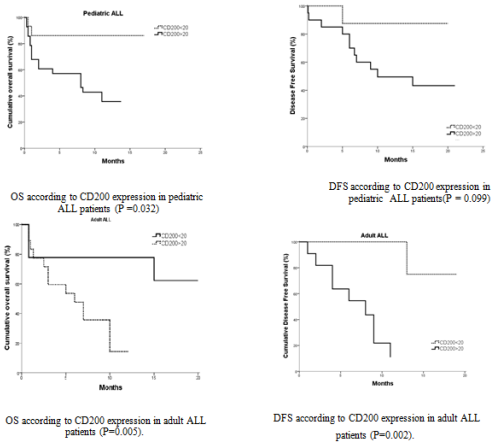


Fig. 6. OS and DFS in ALL patients group (total patient , pediatric ALL, adult ALL)

Results of follow up of responders after induction chemotherapy are summarized in table (6). All of patients with $CD200^+$ and $CD56^+$ cannot respond to therapy, survive and in terms of death had significant differences in induction death or in CR. As well as, the shorter OS and DFS were found in $CD200^+CD56^+$ but the highest OS and DFS achieved by $CD200^-$ $CD56^-$, P<0.001(figure 7, 8).

Table (6). Clinical outcome and OS according to CD200, CD56 positivity in total ALL patients.

	ALL(n=70)								
	CD200- CD56- N=20		**CD200+CD56-** N=43		**CD200- CD56+** N=4		**CD200+ CD56+** N=3		*P*
	N	%	N	%	N	%	N	%	
CR	12	60	28	65.1	1	25	0	0	0.074
PR	1	5	4	9.3	1	25	0	0	0.557
RD	2	10	5	11.6	0	0	0	0	1
ID	4	20	7	16.3	1	25	2	66.7	0.170
Relapse	2	10	10	23.3	2	50	0	0	0.268
Total death	2	10	31	72.1	3	75	3	100	<0.001*
1year cumulative OS (%)	90		27.1		50		0		<0.001*
Mean *(months)*	18.080		9.431		7.875		0.533		
CI 95%	15.556-0.604		6.978-11.885		0.1-16.429		0.249-0.818		
1 year cumulative DFS (%)	100		38.9		66.7		0		<0.001*
Mean *(months)*	11.315		11.761		10.333		0.150		
CI 95%	9.254-12.651		9.054-14.469		4.298-16.369		0.052-0.248		

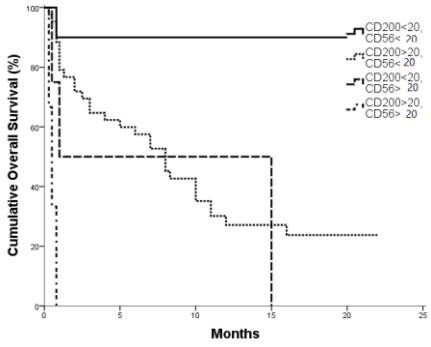


Figure (7). OS in total ALL patients according to CD200 and CD56 positivity

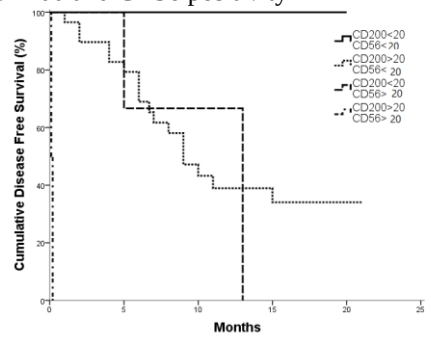


Figure (8). DFS in total ALL patients according to CD200 and CD56 positivity

Discussion

CD200 is a trans- membrane cell surface immunosuppressive glycoprotein which transduces inhibitory signals through interaction with CD200R **(Barclay *et al.*, 2002)**. CD200 can be used as MRD markers in ALL patients and can also can serve as therapy targets **(Adnan Awad *et al.*, 2016)**. CD200 is a key immunosuppressive molecule that has been implicated as a poor prognostic factor in multiple myeloma and acute myeloid leukemia **(Cox *et al.*, 2014)**.

We found in our study, the expression of CD200 was a quite common feature, was present in 67% of cases. These results are consistent with data reported by **(Alapat et al., 2012)** studies in which 19 of 25 cases were CD200 positive. These are 19 of 20 B-ALL (95%) and 5 CD200- T-ALL. While our data listed(37 of 51)B-ALL

cases were positive for CD200 with ratio 72.5 % and(9 of 15) T-ALL cases(60%) were $CD200^+$ which being in partial accordance with the reported data. Furthermore the higher prevalence of CD200 expression in 70% of adult ALL patients in contrast to 65% of pediatric ALL patients.

However, Adnan Awad *et al.*, (2016) reported that CD200 had widely expressed in their studies groups,(80.3%) of B-ALL and can be used as MRD markers ,serve as therapy targets in ALL patients. This ratio also was consistent with our results.

Our results show that CD200 positivity did not correlate with age, sex. Whereas, patients with $CD200^-$ expression (under cut off) was significantly associated with male gender, no previous studies were report in this point. However, demographic or clinical data show that $CD200^+$ expression were significantly associated with Splenomegaly, lower hemoglobin and platelet and did not influence in the clinical outcome .

Expression of CD200 determined by Cox *et al.*,(2014) study were found higher CD200 in bulk ALL cells compared to normal BM cells (p=0.001) which agree with our study that found significant difference in CD200 in ALL patients compared to control cases (P = 0.0001). Thus , our study may confirm utility of CD200 evaluation in distinguishing between leukemic lymphoblasts and normal hematogones that reported by (Alapat *et al.*, 2012). The later study also found that CD200 is significantly over- or under expressed in 55% of $CD34^+$ B-ALLs. Whereas, in this study , the myeloid marker CD34was significantly more frequently expressed in the $CD200^+$ ALL group not regarding to T or B- ALL .

Investigation of CD200 positive leukemic cells to detect its influence on prognosis clear that high express of $CD200^+$ is associated with bad prognosis or negative trend in OS and DFS both in total(whole) patients group ,although statistical significance was not reached for DFS in pediatric ALL patients which may be explains by more CD34+ frequently in CD200+ pediatric ALL patients as it reported before event-free survival was shorter for patients with CD34- leukemia (P = .0014) **(Borowitz *et al.*, 1990)**. In contrast, CD34 expression is associated with features of poor prognosis in adult ALL**(Thomas *et al.*, 1995)**. Up to our knowledge, we are the first to find a statistically significant influences on OS and DFS regarding CD200 positivity.

NK cells usually kill target cells by engagement of specific, pair-wise combinations of receptors and induced secretion of tumor necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma) **(Bryceson *et al.*, 2006)**. It is play an important role in tumor-cell clearance, particularly against leukemia cells which in turn may Reverse natural cytotoxicity receptors (NCRs) phenotype after CR suggested so leukemia cells might be involved in NCR down-regulation **(Fauriat *et al.*, 2007)**. Furthermore CD56 expression has been determined in many hematologic malignancies with adverse influence. Expression of CD56 has been increased at relapse in T-ALL **(Hashimoto *et al.*, 2002)**.

CD56 expression in ALL is uncommon, our data demonstrated that the incidence of positive CD56 expression in the ALL patients was 10% for the whole group patients. Whereas, $CD56^+$ was expressed in pediatric ALL patients with a percent 11.6% and 7.4% in adult ALL patients that similar to rates listed in many literature . CD56 is expressed differentially on adult T-ALL with ratio13.9% by **(Fischer *et al.*, 2009)**. It was detected in 9% in ALL patients reported by **Abdulateef *et al.*, (2014)**, 8% **Ravandi *et al.*,(2002)**, mildly lower frequency of CD56 expression were reported earlier; 3% **Paietta *et al.*, (2001)**,5% **Seegmiller *et al.*, (2009)** and 2.2% **(Hussein *et al.*, 2011).**

However, study of Abdulateef *et al.*, (2014) reported that one of three cases had t(9;22)(BCR/ABL) expressed CD56 which different with our results as none of 6 cases had t(9;22) expressed CD56. Whereas, five of these cases expressed CD200.

CD56+ ALL patients in all studies group exhibited few differences at diagnosis, namely higher platelet count. For other pretreatment characteristics no difference was found that comparable to others who reported lower white blood count, higher platelet levels and CNS involvement **(Fischer *et al.*, 2009)**.

CD56 expression has been shown to be have prognostic relevance in certain

hematologic malignancies **(Suzuki *et al.*, 2003)**. An inferior survival of CD56+ T-ALL was reported by **Montero *et al.*, (2003)**, short survival were observed in patients who expressed CD56 **(Montero *et al* ., 2007)**. Our results demonstrated that the OS was significantly lower in the group expressing CD56 in leukemic blasts, which was compatible with above data. We also found low complete remission and shorter DFS in CD56+ patients that agreement with low remission rates and biological aggressiveness in a significant proportion of acute leukemias were associated with CD56 expression at diagnosis **(Elyamany *et al.*, 2013)**. On the other hand, our results were not in agreement with **Fischer *et al.*, (2009)** who reported that no significant influence on overall (48% *vs.* 59%) and disease free survival (67% *vs.* 57%) at three years.

In this study we examined the presence of both CD200+ , CD56+ and we investigated whether CD56 expression in ALL is a risk factor for shorter OS,DFS and a higher death rate during induction chemotherapy in patients. we found only 3 patients with CD200+,CD56+ and this aberrant CD200 and CD56 expression in ALL at diagnosis has been recognized to be a poor prognostic indicator and associated with biological aggressiveness. However , CD56 expression has been associated with low remission rate.

Conclusions

CD200 and/or CD56 expression in ALL at diagnosis are poor prognostic indicators associated with biological aggressiveness and CD56 expression has been associated with low remission rate..

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الملخص العربي

عنوان البحث: المدلول الأكلينيكى ل CD200 و CD56 فى مرضى سرطان الدم الليمفاوى الحاد

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إن سرطان الدم الليمفاوي الحاد يعد من اكثر أنواع السرطانات انتشارا وقد أصبح الاتجاه في معظم الدراسات هو الحصول على معلومات تفيد في فهم المزيد عن هذا المرض وتحديد درجات استجابة المرضى واي منهم في خطر. بالإضافة الي تقييم المخاطر التي تعتمد على عدد من النتائج السريرية والمخبرية مثل كريات الدم البيضاء والعمر عند التشخيص . لذلك هدفت الدراسة إلى بيان تأثير في مرضى سرطان الدم الليمفاوي الحاد.CD200&CD56النذير لدلالات المجموعة
تم قياس نسبة CD200 وCD56 قبل العلاج الكيميائي؛ لحديثي التشخيص، في 70 عينة من نخاع العظم (BM) و / أو عينات الدم (PB)، بواسطة جهاز الفلوسيتوميتري ، من بينهم 27 حالة سرطان دم ليمفاوي في الكبار و43في الأطفال . سبعة وأربعون من 70 حالة (67٪) أظهرت $^{+}$CD200، 7 حالات (10٪)$^{+}$CD56 و 3 حالات فقط (4.3٪) جمعت بين +CD200 و$^{+}$CD56في حين ان المجموعة الضابطة لم تظهر اي نتائج إيجابية لكلا النذيرين .ثم أظهرت نتائج هذه الدراسة أيضا ان المرضى ذوى +CD200 يعانون من تضخم الطحال وانخفاض هيموجلوبين الدم والصفائح الدموية مع ارتفاع وجود +CD34 هذا بالإضافة الي انخفاض نسبه كلا من البقاء على قيد الحياة والبقاء خاليا من المرض بعد الشفاء في مجموعات الدراسة الايجابية مقارنة بالمجموعات التي لم تظهر CD200. من ناحية أخرى، قد وجد ان المرضى ذوي +CD56لديهم استجابة اقل للعلاج بالإضافة الي انخفاض نسبه البقاء على قيد الحياة والبقاء خاليا دون مرض فترة اطول في حال اتمام الشفاء. اما النسبة القليلة من المرضى الذين أظهروا كلا النذيرين وجد ان لديهم ادنى متوسط بقاء على قيد الحياة وخلو من المرض. تلخص النتائج أن مجموعتي CD200&CD56 في مرضى سرطان الدم الليمفاوي الحاد عند التشخيص هي دلالات لنذير سئ وتكون مصحوبة بأعراض بيولوجية أكثر حدة. علاوة على أن دليل المجموعة CD56. يكون مصحوبًا بمعدل استجابة أقل للعلاج.